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Use of Gamma-Hexachlorocyclohexane (Lindane) to Determine the Ontogeny of Metabolism in the Developing Rat

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16. ABSTRACT

The compound lindane has been used as a model substrate to study the ontogeny of metabolism in the developing Fischer 344 rat. The distribution and metabolic fate of the model substrate at 2, 9, 16, and 23 days of age was investigated following subcutaneous administration of 20 mg/kg of lindane containing 0.5 μCi[U-^{14}C]-lindane in peanut oil. Groups of ten pups (5 male and 5 female) were sacrificed at 4 hr intervals during the 24 hr period following dosing. Adrenals, blood, brain, heart, lung, liver, and kidneys were analyzed for radioactivity. Urine samples were analyzed for radioactivity and metabolites of lindane. There was a significant age dependent increase in the metabolism of lindane in the rat. High levels of radioactivity in the lung and increased reductive dechlorination suggest that the lung may play a greater role in metabolism of lindane by young rats. Oxidative phase I reactions increased significantly while anaerobic reductive dechlorination of lindane to 4-chlorophenylmercapturic acid decreased significantly with age. Phase II sulfate and glutathione conjugations decreased significantly and glucuronide conjugation increased significantly with age. Metabolism and excretion of the model substrate appears to parallel development of the hepatic enzymes involved in phase I and phase II reactions.

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USE OF γ-HEXACHLOROCYCLOHEXANE (LINDANE) TO DETERMINE THE ONTOGENY OF METABOLISM IN THE DEVELOPING RAT

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The compound lindane (γ-hexachlorocyclohexane) has been used to study the ontogeny of metabolism in the developing Fischer 344 rat. The distribution and metabolic fate of lindane at 2, 9, 16, and 23 d of age was investigated following subcutaneous administration of lindane at 20 mg/kg containing 0.5 μCi [U-^{14}C]lindane in peanut oil. Groups of 10 pups (5 male and 5 female) were sacrificed at 4-h intervals during the 24-h period following dosing. Adrenals, blood, brain, heart, lung, liver, and kidneys were analyzed for radioactivity. Urine samples were analyzed for radioactivity and metabolites of lindane. There was a significant age-dependent increase in the metabolism of lindane in the rat. High levels of radioactivity in the lung and increased reductive dechlorination suggest that the lung may play a greater role in metabolism of lindane by young rats. Oxidative phase I reactions increased significantly, while anaerobic reductive dechlorination of lindane to 4-chlorophenylmercapturic acid decreased significantly with age. Phase II sulfate and glutathione conjugations decreased significantly and glucuronide conjugation increased significantly with age. Metabolism and excretion of lindane appear to parallel development of the hepatic enzymes involved in phase I and phase II reactions.

INTRODUCTION

Lindane (γ-hexachlorocyclohexane, γ-HCH) is still widely used as an agricultural pesticide, especially in developing countries, and on humans for treatment of lice and scabies infestations (Lange et al., 1981; Grey et al., 1983; Davies et al., 1983). Children and young animals appear to be particularly sensitive to lindane toxicity (Ginsburg et al., 1977; Hanig et al., 1976; Solomon, 1977). Therefore, it is important to study the metabolism of lindane in the developing animal.

There are few detailed studies on metabolism of foreign compounds in the developing animal. The metabolism of foreign com-

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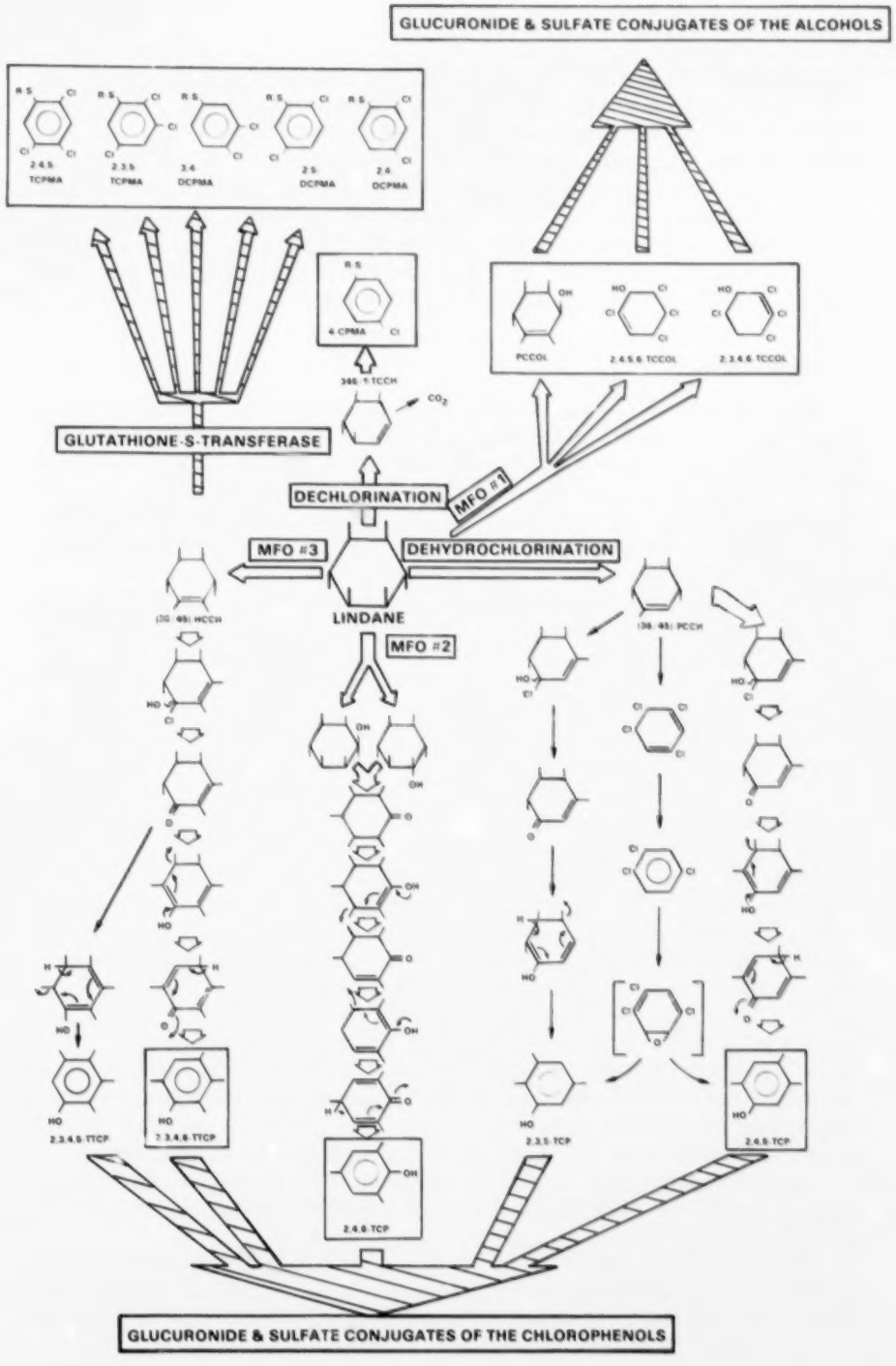
pounds occurs principally in the liver. Foreign compounds undergo a wide range of metabolic transformations, many of which are catalyzed by the hepatic microsomal enzymes located in the smooth endoplasmic reticulum (SER). Synthesis of enzymes occurs in the rough reticulum, which when saturated with enzyme appears to lose its ribosomes and becomes SER. The SER, absent during the early fetal period of the rat, appears for the first time on gestation d 20 (Chedid and Nair, 1974) and progressively increases. Biochemical and morphologic studies have shown that fetal and newborn animals have a deficient hepatic microsomal drug-metabolizing system, which increases after parturition and reaches adult levels within 3–8 wk (Kato et al., 1964; Yaffe et al., 1968; Feuer and Liscio, 1970; Short et al., 1976; MacLeod et al., 1972; Pelkonen et al., 1973; Rane and Sjoqvist, 1972).

In this study the distribution, metabolism, and excretion of γ-hexachlorocyclohexane (lindane) in the developing rat was studied. Metabolism of lindane (Fig. 1) involves multiple pathways, which includes both phase I and phase II pathways involved in the biotransformation of foreign compounds. The biotransformation of lindane to 2,4,6-trichlorophenol (2,4,6-TCP) and 2,3,4,6-tetrachlorophenol (2,3,4,6-TTCP) are catalyzed by cytochrome P-450 through distinctly different mechanisms, with the first step being hydroxylation and dehydrogenation, respectively (Chadwick et al., 1975; Tanaka et al., 1977; and Stein et al., 1977), while oxidation to the alcohols may involve nucleophilic attack of the superoxide anion (Tanaka et al., 1979). Dehydrochlorination of lindane through pentachlorocyclohexene leads to 2,4,5-trichlorophenol (2,4,5-TCP) (Tanaka et al., 1977), while reductive dechlorination, an anaerobic reaction, is the rate-limiting step in the metabolism of lindane through tetrachlorocyclohexene to 4-chlorophenylmercapturic acid (4-CPMA) and carbon dioxide (Kurihara et al., 1979; Chadwick et al., 1978; Matsumura et al., 1976). Different isozymes are probably involved in the conjugation of the chlorophenol and alcohol metabolites. Multiple forms of sulfotransferase (Paulson, 1976) and UDP-glucuronyltransferase (Watkins et al., 1982; Boutin et al., 1984, 1985) are found in the liver, and it has been reported that glucuronidation of planar molecules like the chlorophenols occurs via a different isozyme of UDP-glu-

FIGURE 1. The biotransformation of lindane in rats. Where the configuration of the metabolites is known, this is indicated by a fractional notation, numbers in the numerator denoting chlorine above and numbers in the denominator denoting chlorine below the plane of the ring. Abbreviations: 2,4,6-TCP, 2,4,6-trichlorophenol; 2,4,5-TCP, 2,4,5-trichlorophenol; 2,3,5-TCP, 2,3,5-trichlorophenol; 2,3,4,6-TTCP, 2,3,4,6-tetrachlorophenol; 2,3,4,5-TTCP, 2,3,4,5-tetrachlorophenol; 2,3,4,6-TCCOL, 2,3,4,6-tetrachloro-2-cyclohexen-1-ol; 2,4,5,6-TCCOL, 2,4,5,6-tetrachloro-2-cyclohexen-1-ol; PCCOL, 2,3,4,5,6-pentachloro-2-cyclohexen-1-ol; 346/5-TCCH, γ-3,4,5,6-tetrachlorocyclohex-1-ene; (36/45)-HCCH, γ-1,2,3,4,5,6-hexachlorocyclohexene; (36/45)-PCCH, 2,3,4,5,6-pentachlorocyclohexene; TCPMA, trichlorophenylmercapturic acid; DCPMA, dichlorophenylmercapturic acid; 4-CPMA, 4-chlorophenylmercapturic acid; MFO, mixed-function oxidase.


GLUCURONIDE & SULFATE CONJUGATES OF THE ALCOHOLS
2,4,5-TCPMA
2,3,5-TCPMA
3,4-DCPMA
2,5-DCPMA
2,4-DCPMA
4-CPMA
PCCOL
2,4,5,6-TCCOL
2,3,4,6-TCCOL
GLUTATHIONE-S-TRANSFERASE
DECHLORINATION
MFO #1
MFO #3
DEHYDROCHLORINATION
LINDANE
MFO #2
2,3,4,6-TTCP
2,3,4,5-TTCP
2,4,6-TCP
2,3,5-TCP
2,4,5-TCP
GLUCURONIDE & SULFATE CONJUGATES OF THE CHLOROPHENOLS

curonyltransferase than glucuronidation of the monoterpenoid alcohols, which, like the lindane-derived alcohol metabolites, are bulkier than the phenols (Boutin et al., 1984). Phase I reactions of lindane metabolism are indicated by total excreted alcohols, 2,4,6-trichlorophenol, 2,3,4,6-tetrachlorophenol, 2,4,5-trichlorophenol, and 4-chlorophenylmercapturic acid. Phase II reactions include UDP-glucuronyltransferase, indicated by chlorophenol and alcohol glucuronides; sulfotransferase, indicated by chlorophenol and alcohol sulfates; and glutathione *S*-transferase, represented by total mercapturic acids.

METHODS

One hundred timed pregnant Fischer 344 rats (Charles River Breeding Laboratories, Kingston, N.Y.) were housed singly in polycarbonate cages with free access to food (Purina Lab Chow, Ralston Purina Co., St. Louis, Mo.) and water throughout pregnancy. Light was maintained on a 12:12 light–dark cycle and room temperature was maintained at 22 ± 2°C. Bedding was heat-treated pine shavings (Northeastern Products Corp., Warrensburg, N.Y.).

Dams were allowed to give birth, and only pups born within 24 h of the first signs of parturition were used in this study. Pups aged 2, 9, 16, and 23 d were dosed sc in the nape of the neck with 20 mg lindane (containing 0.5 μCi [U-^{14}C]lindane per kilogram body weight in peanut oil and placed back in the cage with the dam. The unlabeled lindane (>99% γ-hexachlorocyclohexane) was obtained from the Environmental Protection Agency Health Effects Research Laboratory Analytical Reference Standards Repository, Research Triangle Park, N.C. [U-^{14}C]Lindane with a specific activity of 46 mCi/mmol and a radiochemical purity of 98% was obtained from Amersham, Arlington Heights, Ill. Littermates were used throughout the study. Groups of 10 pups (5 males and 5 females) were voided and sacrificed at 4-h intervals during the 24-h period following dosing. At the beginning of the 4-h interval the pups were removed from the mother, and their bladders were voided by perineal stimulation (Kavlock and Gray, 1982). The 2- and 9-d-old pups were then placed in beakers containing bedding and placed in a water bath maintained at 40–45°C. The 16- and 23-d-old pups were then placed in glass funnels set up for urine collection at room temperature. At the end of the 4-h interval the pups were voided again, and urine volume was recorded and saved for analysis. Blood samples from the descending aorta were taken for determination of radioactivity. The pups were sacrificed by decapitation, and brain, lung, heart, liver, kidneys, and adrenals were taken for determination of radioactivity.

Blood, brain, lung, heart, liver, kidneys, and adrenals were weighed and combusted in a Packard Tri-Carb model 306 sample oxidizer. The radioactivity was determined in a Packard Tri-Carb model

3380 liquid scintillation spectrometer employing a quench correction curve.

Urine samples of less than 1 ml were taken to 1 ml with high-performance liquid chromatography (HPLC) grade distilled water (Fisher Scientific Co., Fair Lawn, N.J.). The 1-ml urine samples were acidified (pH 2.0) with concentration HCl and extracted twice with 2-ml aliquots of isooctane (Burdick and Jackson Laboratories, Inc., Muskegon, Mich.) for determination of free chlorophenols, alcohols, and unaltered lindane. The residual urines were hydrolyzed with β-glucuronidase (Sigma Chemical Co., St. Louis, Mo.) by the method of Rickert and Long (1981) and, after acidification, were extracted twice with 2-ml aliquots of isooctane to obtain the chlorophenol and alcohol glucuronides. Residual urines from this extraction were hydrolyzed with arylsulfatase (Sigma Chemical Co., St. Louis, Mo.) by the method of Rickert and Long (1981) and, after acidification, were extracted twice with 2-ml aliquots of isooctane to obtain the chlorophenol and alcohol sulfates. Residual urines from this extraction were hydrolyzed and acylated as described by Allsup and Walsh (1982) and extracted twice with 5-ml aliquots of isooctane for determination of the mercapturic acid metabolites of lindane. Urine extracts were analyzed for lindane metabolites on a Tracor model MT 222 gas chromatograph equipped with a ^{63}Ni electron-capture detector and linearizer. Output from the chromatograph was compiled and recorded in a usable format by a Tracor 812 analytical processor. The column consisted of a 1.8 m × 4 mm ID glass U-tube packed with 5% ethylene glycol succinate (EGS) + 1% H_3PO_4 on 90/100 mesh Anakrom A (Analabs, North Haven, Conn.). The column was maintained isothermally at 170°C. Inlet and detector temperatures were 225°C and 330°C, respectively. The 5% methane/argon carrier gas was regulated at 55 cm^3/min. Radioactivity in urine and urine extracts was analyzed directly in a Packard Tri-Carb model 3380 liquid scintillation spectrometer using a quench correction curve and a scintillation solution containing 500 ml toluene, 500 ml cellosolve, 5 g 2,5-diphenyloxazol (PPO), and 50 mg 1,4-bis-2(5-phenoxazolyl)benzene (POPOP).

Data were analyzed by the general linear models procedure with a computer-based statistical analysis system (Ray, 1982). Two-way analysis of variance by age and sex showed no sex differences, so the data were pooled across sex for analysis. If there was no interaction, Duncan's multiple-range test (Duncan, 1955) was used to interpret the data from this study. Differences were considered significant at $p < 0.05$ unless otherwise stated.

RESULTS

The blood levels of lindane-derived radioactivity at the end of the 4-h intervals were significantly greater in the 2-d-old pups than in the

9-, 16-, and 23-d-old pups (Fig. 2). There was a fourfold decrease in the average radioactivity in the blood of the 23-d-old pups compared to the 2-d-old pups.

There were no significant differences in the amount of radioactivity in the heart and adrenals throughout the study. The distribution of radioactivity in the brain, lung, liver, and kidney is shown in Fig. 3. The amount of radioactivity in the brain and lungs of the 2- and 9-d-old pups was significantly greater than in the 16- and 23-d-old pups (Fig. 3, *a* and *b*). Brain and lung radioactivity in the 2-d-old pups was 2.8 and 3.7

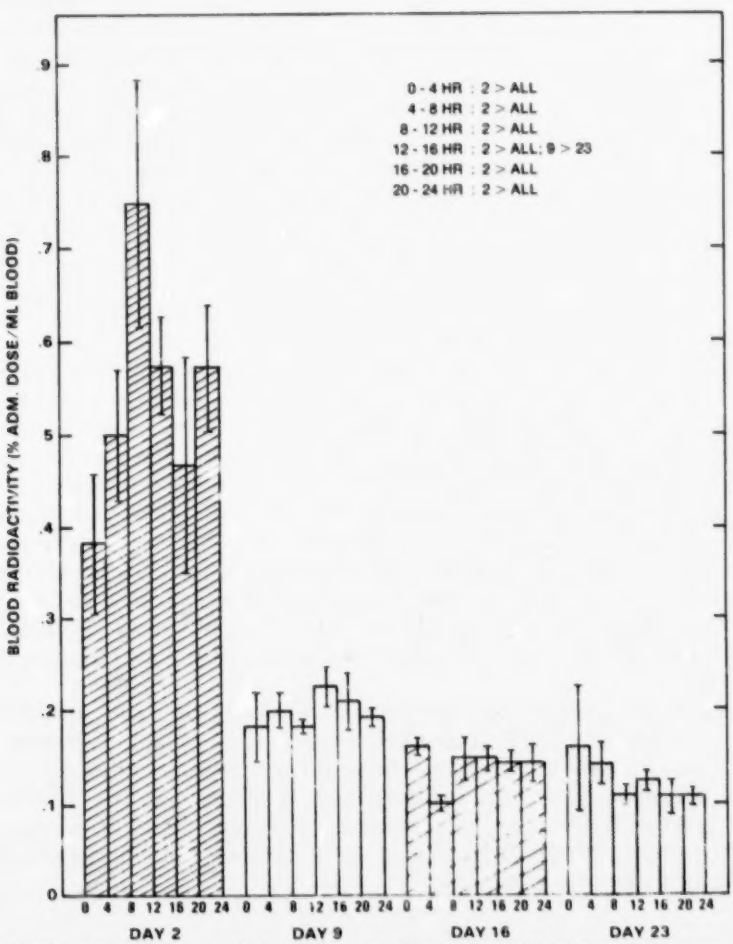


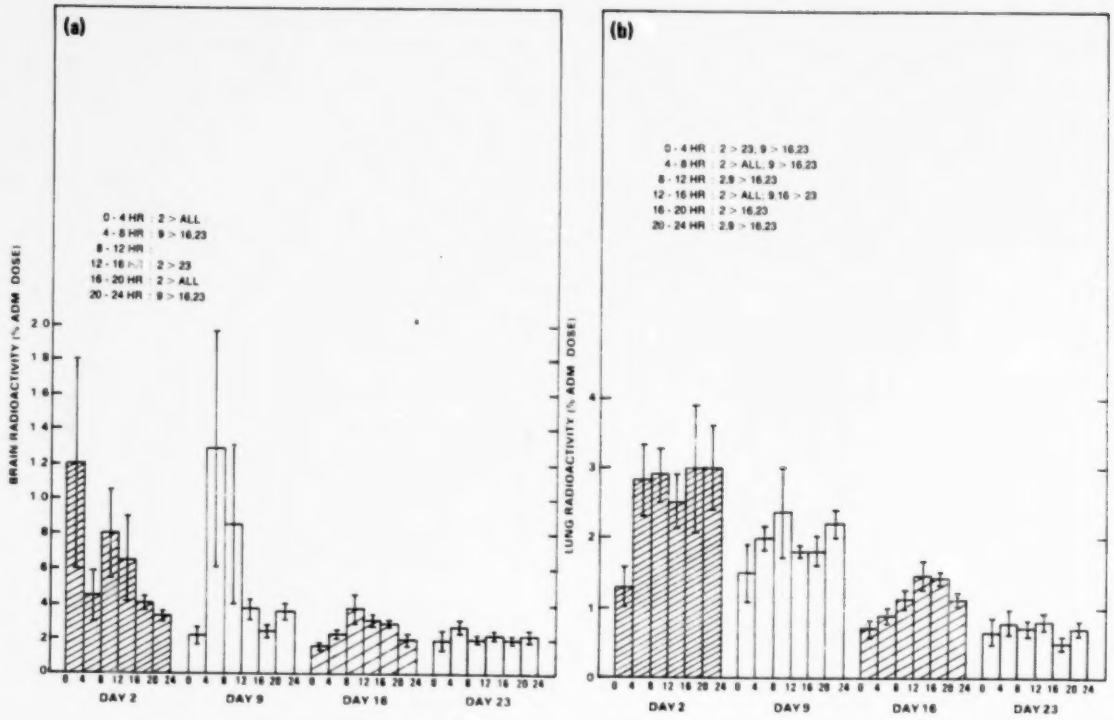
FIGURE 2. The effect of age on lindane-derived radioactivity in blood. Each bar represents the mean percent administered dose per milliliter blood for 10 rats. The vertical lines indicate the SE. Comparisons of pup ages in each time segment are given at top.

times greater than in the 23-d-old pups, respectively. Radioactivity in the liver of 2-, 16-, and 23-d-old pups was significantly greater than in the 9-d-old pups (Fig. 3*c*). The levels of radioactivity in the kidneys of 16- and 23-d-old pups were significantly greater than that in the 2- and 9-d-old pups (Fig. 3*d*). The 23-d-old pups had kidney radioactivity levels 1.6 times greater than those of the 2-d-old pups.

The effect of age on the urinary excretion of lindane-derived radioactivity is presented in Fig. 4. The lindane-derived radioactivity excreted in each 4-h interval is shown in Fig. 4*a;* however, since the rats were voided at the beginning and end of the 4-h intervals to ensure that the radioactivity would represent that interval and since littermates were used for the 4-h intervals, we have summed the urinary radioactivity and metabolites across the 6 4-h intervals to get a 24-h urinary excretion (Fig. 4*b;* also see Tables 1 and 2). The excretion of lindane-derived radioactivity increased significantly with age (Fig. 4*b*). The lindane-derived urinary radioactivity in the 23-d-old pups was double that in the 2-d-old pups.

The effect of age on the distribution of radioactivity in the 4 urinary metabolite fractions is summarized in Fig. 5. Studies in our laboratory with adult Fischer 344 rats (35 d old) has shown the distribution of radioactivity in the free, glucuronide, sulfate, and polar fractions to be 27.4 ± 2.9, 8.4 ± 0.8, 10.3 ± 1.3, and 53.9 ± 1.3, respectively (unpublished data). The proportion of free metabolites (Fig. 5*a*) increased significantly with age, reaching the level seen in adults by d 16. The proportion excreted as glucuronides started out higher than in adults, increased significantly, reaching a peak in the 16-d-old pups, and decreased significantly in the 23-d-old pups but still was higher than in the adults (Fig. 5*b*). The proportion of sulfate metabolites (Fig. 5*c*) was higher in the 2-d-old pups than in the adult, decreased significantly with age to a low in the 16-d-old pups, and then increased significantly in the 23-d-old pups, approaching the level seen in the adults. The polar metabolites were significantly lower in the 16-d-old pups but then increased in the 23-d-old pups, so that the value obtained was not significantly different from that of the 2- and 9-d-old pups (Fig. 5*d*).

The effect of age on phase I reactions is shown in Table 1. There was no significant difference in total alcohol excretion throughout the study. With increasing age, the excretion of total 2,4,6-trichlorophenol, total 2,3,4,6-tetrachlorophenol, and total 2,4,5-trichlorophenol increased significantly, while excretion of 4-chlorophenylmercapturic acid by d 23 had decreased significantly. The effect of age on phase II reactions is shown in Table 2. There was a significant decrease in alcohol glucuronides between the 2- and 9-d-old pups, which then increased significantly with age such that the 23-d-old pups were not significantly different from the 2-d-old pups. The alcohol sulfates decreased significantly with age in the 9- and 16-d-old pups and then


(a)
0 - 4 HR : 2 > ALL
4 - 8 HR : 9 > 16,23
8 - 12 HR :
12 - 16 HR : 2 > 23
16 - 20 HR : 2 > ALL
20 - 24 HR : 9 > 16,23
BRAIN RADIOACTIVITY (% ADM DOSE)
DAY 2
DAY 9
DAY 16
DAY 23
(b)
0 - 4 HR : 2 > 23, 9 > 16,23
4 - 8 HR : 2 > ALL, 9 > 16,23
8 - 12 HR : 2,9 > 16,23
12 - 16 HR : 2 > ALL, 9,16 > 23
16 - 20 HR : 2 > 16,23
20 - 24 HR : 2,9 > 16,23
LUNG RADIOACTIVITY (% ADM DOSE)
DAY 2
DAY 9
DAY 16
DAY 23

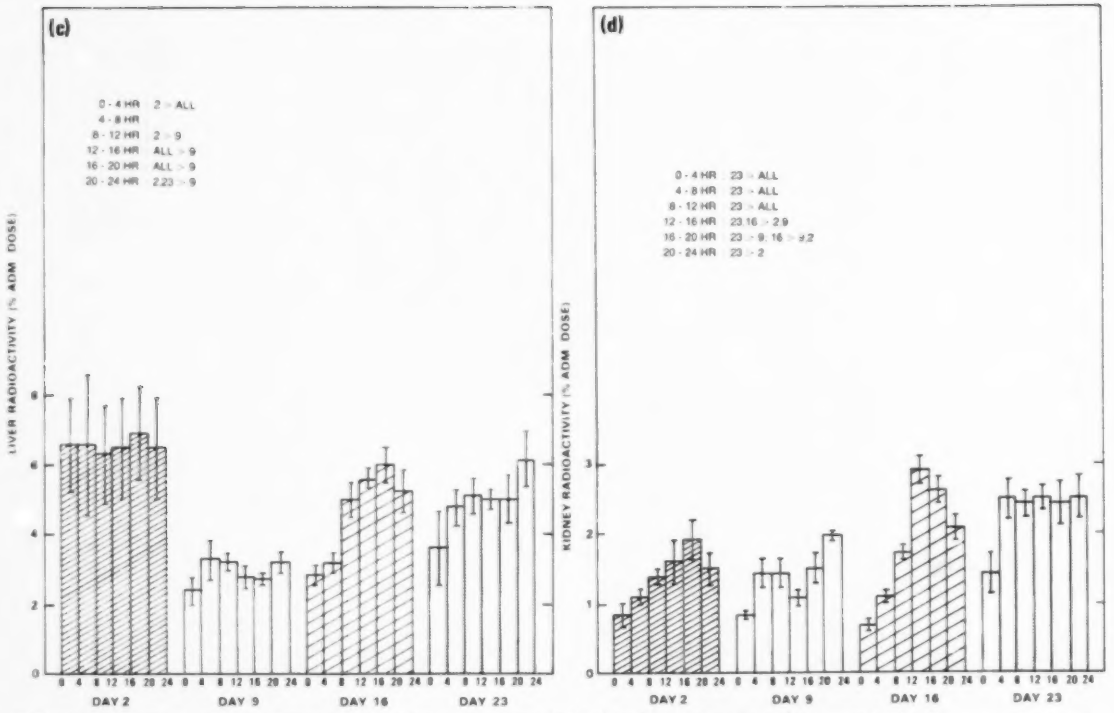

(c)
0 - 4 HR 2 > ALL
4 - 8 HR
8 - 12 HR 2 > 9
12 - 16 HR ALL > 9
16 - 20 HR ALL > 9
20 - 24 HR 2,23 > 9
LIVER RADIOACTIVITY (% ADM DOSE)
0 4 8 12 16 20 24
DAY 2
DAY 9
DAY 16
DAY 23
(d)
0 - 4 HR 23 > ALL
4 - 8 HR 23 > ALL
8 - 12 HR 23 > ALL
12 - 16 HR 23,16 > 2,9
16 - 20 HR 23 > 9, 16 > 9,2
20 - 24 HR 23 > 2
KIDNEY RADIOACTIVITY (% ADM DOSE)
0 4 8 12 16 20 24
DAY 2
DAY 9
DAY 16
DAY 23

FIGURE 3. Effect of age on tissue distribution of lindane-derived radioactivity. Each bar represents the mean percent administered dose/organ for 10 rats. Brain, lung, liver, and kidney are shown in (*a*), (*b*), (*c*), and (*d*), respectively. The vertical lines indicate the SE. Ages of pups are compared at top.

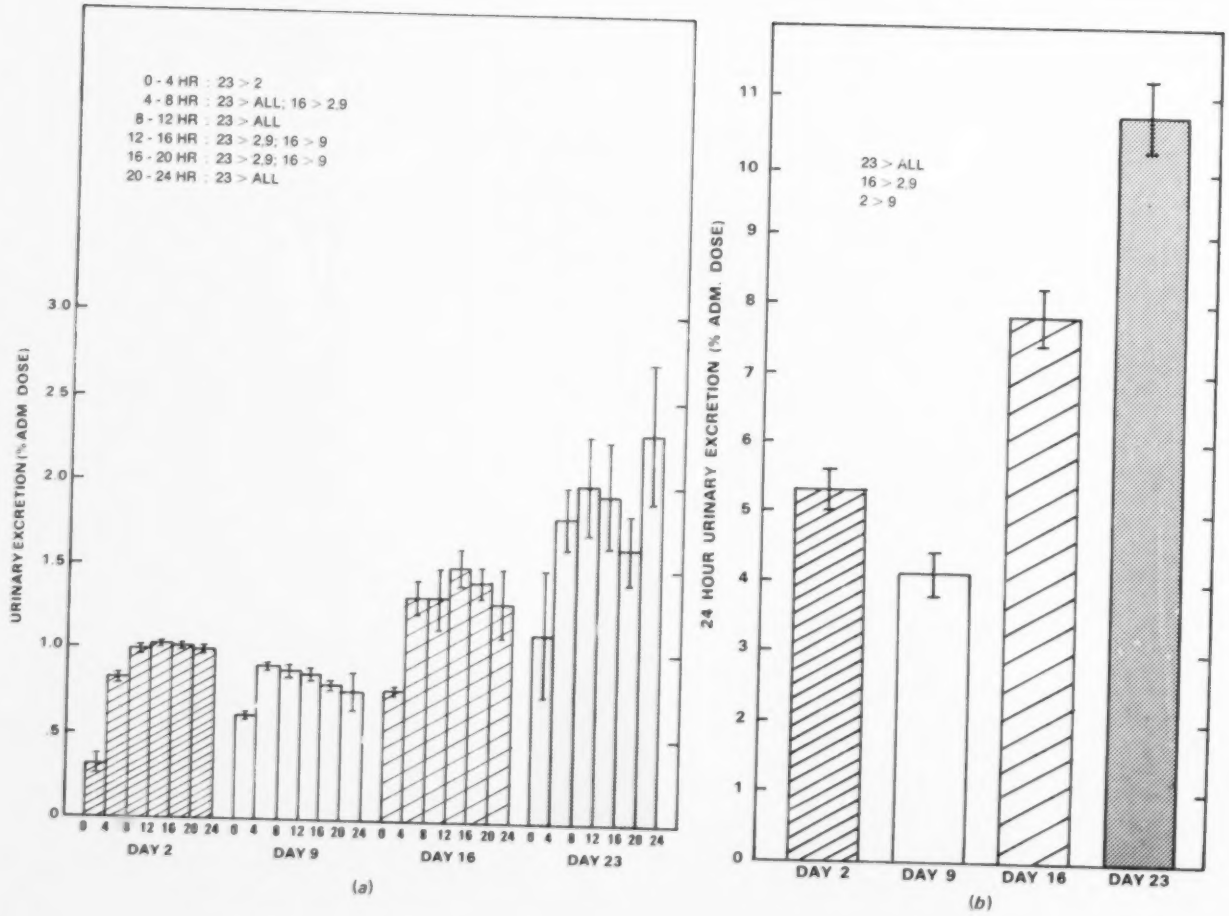

0 - 4 HR : 23 > 2
4 - 8 HR : 23 > ALL; 16 > 2,9
8 - 12 HR : 23 > ALL
12 - 16 HR : 23 > 2,9; 16 > 9
16 - 20 HR : 23 > 2,9; 16 > 9
20 - 24 HR : 23 > ALL
URINARY EXCRETION (% ADM DOSE)
0
.5
1.0
1.5
2.0
2.5
3.0
0 4 8 12 16 20 24
DAY 2
DAY 9
DAY 16
DAY 23
(a)
24 HOUR URINARY EXCRETION (% ADM. DOSE)
23 > ALL
16 > 2,9
2 > 9
0
1
2
3
4
5
6
7
8
9
10
11
DAY 2
DAY 9
DAY 16
DAY 23
(b)

TABLE 1. Effect of Age on Phase I Reactions[a]

Age (d)	Total alcohols	Total 2,4,6-tri-chlorophenol	Total 2,3,4,6-tetra-chlorophenol	Total 2,4,5-tri-chlorophenol	Total 4-chlorophenyl mercapturic acid
2	7.46 ± 1.42	13.48 ± 1.09	6.93 ± 0.67	7.21 ± 0.63	6.64 ± 0.85
9	5.65 ± 0.62	25.42 ± 1.19	13.61 ± 0.45	7.49 ± 0.49	11.28 ± 0.71
16	7.05 ± 0.73	27.76 ± 1.04	20.43 ± 0.48	9.98 ± 0.40	5.26 ± 0.42
23	6.89 ± 0.52	31.21 ± 0.74	21.87 ± 0.77	11.67 ± 0.22	2.00 ± 0.15
Age comparison[b]	NS	23 > All 16, 9 > 2	23, 16 > 9, 2 9 > 2	23 > All 16 > 2, 9	9 > All 2, 16 > 23

[a] Values are mean percent of total urinary metabolites ± SE (n = 10).

[b] Comparisons were considered significantly different at $p < 0.05$ by Duncan's multiple-range test. NS indicates the comparisons were not significantly different at $p < 0.05$.

increased significantly in the 23-d-old pups. The chlorophenol glucuronides increased significantly with age while the chlorophenol sulfates decreased significantly with age. Glutathione *S*-transferase activity indicated by total mercapturic acids was significantly greater in the 2- and 9-d-old pups than in the 16- and 23-d-old pups. The percent of total urinary metabolites excreted as unaltered lindane was significantly higher in the 2-d-old pups (2.28 ± 0.29) than in the 9- (0.39 ± 0.10), 16- (0.25 ± 0.05), and 23-d-old (0.33 ± 0.08) pups.

TABLE 2. Effect of Age on Phase II Reactions[a]

	Glucuronidation		Sulfate conjugation		Glutathione conjugation: total
Age (d)	Alcohol glucuronides	Chlorophenol glucuronides	Alcohol sulfates	Chlorophenol sulfates	mercapturic acids
2	1.39 ± 0.28	10.06 ± 1.30	3.35 ± 0.44	25.01 ± 7.14	36.15 ± 3.72
9	0.30 ± 0.02	11.79 ± 1.45	1.60 ± 0.40	12.54 ± 1.35	37.83 ± 1.51
16	0.66 ± 0.10	14.27 ± 1.38	0.86 ± 0.24	13.55 ± 1.25	12.19 ± 0.58
23	1.39 ± 0.11	19.04 ± 1.30	2.09 ± 0.28	8.75 ± 0.66	7.28 ± 0.29
Age comparisons[b]	2, 23 > 16, 9	23 > All 16 > 2	2 > All 23 > 16	2 > All	9, 2 > 16, 23

[a] Values are mean percent of total urinary metabolites ± SE (n = 10).

[b] Comparisons were considered significantly different at $p < 0.05$ by Duncan's multiple-range test.

FIGURE 4. Effect of age on the excretion of lindane-derived radioactivity. (a) Each bar represents the mean percent administered dose excreted per 4 h for 10 rats. (*b*) The 4-h excretions have been summed over the 6 intervals as described in the text, and each bar represents the mean percent administered dose excreted per 24 h (n = 10). The vertical lines indicate the SE. Comparisons of pup ages are given at top.

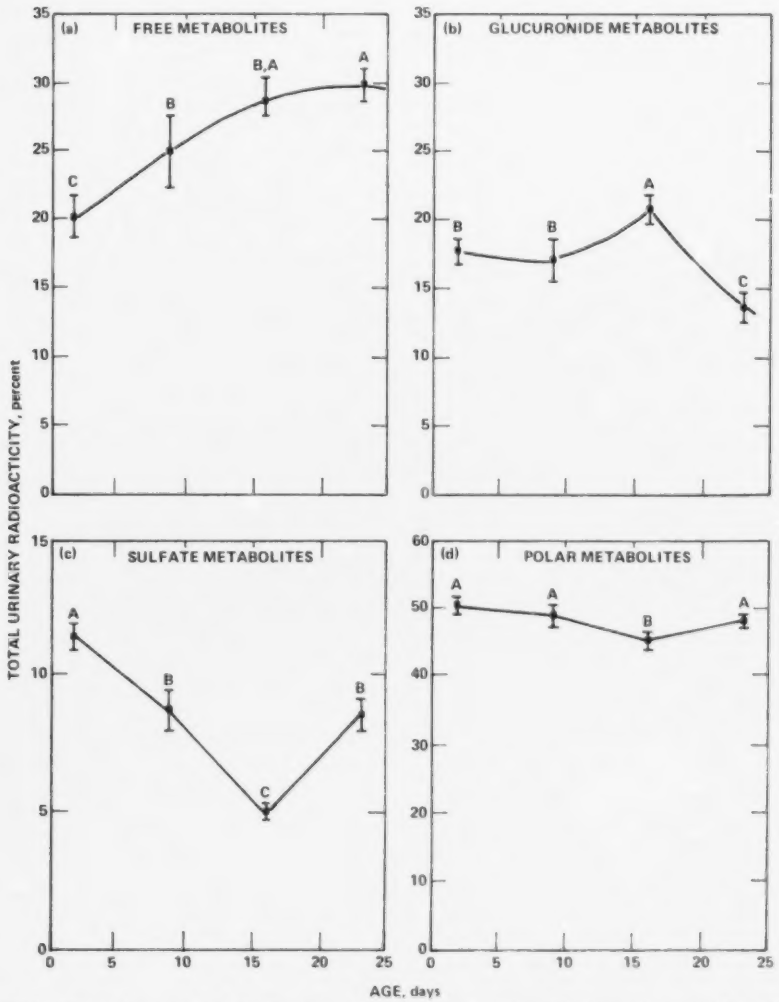


FIGURE 5. Effect of age on the distribution of radioactivity in urine. The free, glucuronide, sulfate, and polar (mercapturic acid) metabolites were extracted and analyzed as described in the methods section. Each point represents the mean percent total urinary radioactivity (n = 10). The vertical lines indicate the SE. Values with the same letter are not significantly different from one another at $p < 0.05$.

DISCUSSION

Several investigators have reported that metabolic activity is low in the newborn rat and increases progressively after birth, reaching a maximum in 3–8 wk and then gradually decreasing with aging (Kato et

al., 1964; MacLeod et al., 1972). Results from the present study indicate that metabolism of lindane in Fischer 344 rats is low at birth and increases significantly with age. Aging resulted in a significantly reduced level of lindane-derived radioactivity in the blood, reduced levels of radioactivity in the brain and lung, increased level of radioactivity in the kidney, increased oxidative phase I reactions, increased excretion of lindane-derived radioactivity, and reduced excretion of unaltered lindane.

The significantly higher levels of radioactivity in the lungs of the 2- and 9-d-old pups and the significantly lower urinary excretion of lindane-derived radioactivity suggest that the lung may be a site of lindane metabolism and that expired air could be a major route of excretion in the young rat. This is further supported by the significantly greater anaerobic reductive dechlorination through tetrachlorocyclohexene to 4-chlorophenylmercapturic acid in the 2-, 9-, and 16-d-old pups. Anaerobic dechlorination to tetrachlorocyclohexene has been shown to be the rate-limiting step in the metabolism of lindane to carbon dioxide (Matsumura et al., 1976; Chadwick et al., 1978).

The distribution of lindane-derived urinary metabolites changes significantly with age. Results from the present study indicate that oxidative phase I reactions increase significantly with age. This agrees with work published by Gillette and Stripp (1975), which states that the cytochrome P-450 mixed-function oxidase system is low at birth and increases after parturition, reaching adult levels in 3–8 wk. The anaerobic dechlorination of lindane through tetrachlorocyclohexene to 4-chlorophenylmercapturic acid decreases significantly with age. The dechlorination step is a reduction pathway, and Yaffe and Juchau (1974) report that both reduction and sulfation are well developed at birth, with in vitro activities in the adult range. Several investigators have reported that the phase II conjugation reactions usually surge around birth. Lucier (1978) and Mannering (1985) reported that UDP-glucuronyltransferase activity for late fetal substrates reaches adult values between d 16 and 20 of gestation and surpasses adult values at birth. In the present study, the urinary glucuronide metabolite fraction is greater than that seen in adult rats. The chlorophenol glucuronides increase significantly with age, while the alcohol glucuronides are high in the 2-d-old pups but decrease significantly in the 9- and 16-d-old pups, with levels in 23-d-old pups increasing so they are not different from 2-d-old pups. Mannering (1985) reported that sulfate conjugation in laboratory animals appears to surge just before or after birth. The urinary sulfate metabolite fractions are higher in 2-d-old pups than adults but lower than adults by d 9. The alcohol and chlorophenol sulfates decrease significantly with age. The alcohol and chlorophenol sulfates are 2.5 times greater than the alcohol and chlorophenol glucuronides in the 2-d-old pups. This agrees with Dutton (1978), who reported that the neonate

tends to excrete drugs preferentially as sulfates, which suggests that sulfation is more developed in the young rat than glucuronidation. Dutton (1982) reported that glutathione *S*-transferases in laboratory animals surge at birth, which agrees with the present study where glutathione conjugation in the 2- and 9-d-old pups is significantly greater than in the 16- and 23-d-old pups.

Boutin and co-workers (1984) reported that glucuronidation of planar molecules such as chlorophenols was more rapid and occurred via a different isozyme of UPD-glucuronyltransferase than the monoterpenoid alcohols, which, like the lindane-derived alcohol metabolites, are bulky. Results from the present study agree with Boutin's work in that the glucuronidation of chlorophenols is greater than that of the alcohols.

In summary, results from this study in Fischer 344 rats indicate that (1) the metabolism of lindane is low at birth and increases significantly with age; (2) the lung may play a greater role in the metabolism of lindane in young rats; (3) oxidative phase I reactions increase significantly with age, while reductive dechlorination decreased significantly with age; (4) phase II reactions are age-dependent, with sulfate and glutathione conjugation decreasing and glucuronide conjugation increasing significantly with age; (5) different isozymes may be involved in glucuronidation of the alcohol and chlorophenol metabolites of lindane.

Studies of this nature may help investigators to evaluate hepatic function in the developing animal. The postnatal metabolism of lindane may aid in the identification of other compounds that, after in utero exposure, may alter the ability of the neonate to biochemically deal with xenobiotics and may also determine the affected metabolic pathways.

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